Spatial Influences on Rates of Denitrification in Floridan Karst Aquifers

By

Colin Geisenhoffer

Dr. James Heffernan, Adviser

May 2014

Master project submitted in partial fulfillment of the

requirements for the Master of Environmental Management degree in

the Nicholas School of the Environment of Duke University

Abstract

Nitrogen is a major contaminant of waterways throughout the world. This nitrogen is derived from a variety of sources including fertilizers, municipal wastewater, and atmospheric deposition. There has been a historical focus on nitrogen loading in surface waters, but in recent times this focus has shifted to denitrification. Denitrification is a major processes in which nitrogen is removed from waterways especially aquifers, which are often low in dissolved oxygen a critical factor for denitrification. This study focuses on the spatial relationships that influence this process using dissolved oxygen, nitrogen, and $\delta^{15}N\text{-}NO_3^-$ data collected from a previous study in the karst Floridan Aquifer System by Heffernan et al. (2012). This data is compared to and various spatial datasets in order to understand their influence on these parameters.

Many previous research studies have already shown how land covers exports varying amounts of nitrogen. Changes in nitrogen export between various landuses will change the amount of nitrogen entering a waterway. This study identified a relationship between landuse and different rates of dissolved oxygen, nitrogen loading, and denitrification. This paper also identified a strong relationship between wastewater treatment plant discharge that is land applied to changes in nitrogen loading and denitrification. To add further credence to the relationships between landuse and nitrogen a comparison of the isotope data collected in the Heffernan et al. (2012) was conducted. This comparison identified particular that various landuses show trends regarding their isotopic composition.

Introduction

Anthropogenic activities have fundamentally altered the nitrogen cycle leading to increases in nitrogen contamination of aquatic ecosystems (Galloway et al. 2010). This increase in nitrogen is partially due to the Haber Boch process and the resulting application of nitrogen fertilizers to agricultural land, as well as wastewater treatment plant effluent. This nitrogen as nitrate (NO_3^-) is transported into aquifers though groundwater flows.

Within aquifers, nitrogen is exposed to anaerobic (reducing) conditions associated with groundwater. These conditions facilitate denitrification, removing some or nearly all of the nitrogen. For example, within the Karst Aquifers of Florida denitrification is highly variable and ranges from 0-97% of $NO_3^-\text{-}N$ being removed (Heffernan et al. 2012). This variation in nitrogen removal may be attributable to differences in the amount of nitrogen loading, aquifer geological structure, or dissolved oxygen concentrations, as well as a variations in the type of electron donors although both heterotrophic and autotrophic receptors are typically both present (Korom 1992). This variability along with the ability of denitrification to alter the isotopic composition of nitrogen species makes determining the source of nitrogen contamination difficult.

It is possible that varying landuses are influencing the rates of nitrogen loading and subsequent denitrification within aquifers. The focus of this study is to address these concerns. Differences arise when comparing nitrate found in groundwater from both pristine and developed lands (Cole et al. 2006). Furthermore, wastewater disposal though sprayfield applications has been identified as a source of contamination for the Upper Floridan Aquifer. In Wakulla Springs, isotopically identified wastewater nitrate as well as conservative tracers were identified downgradient of the sprayfields (Katz et al. 2009). This indicates that wastewater treatment system this may influence nitrogen cycling. By identifying the various sources of nitrogen and comparing any landuses to which these changes are related one can infer that these are leading factors in nitrogen removal.

This study focuses on the spatial relationships that influence this process using dissolved oxygen, nitrogen, and $\delta^{15}N\text{-}NO_3^-$ data collected from a previous study by Heffernan et al. (2012). This study uses the same spring and springsheds to gain a better understanding of landuses influence on these parameters. This study area is a karst system. Karst aquifers are created through the dissolution of limestone by groundwater creating pockets of water that are often connected to one another through various fissures in the rock. These features allow for more complete mixing as compared to other aquifers, which in turn may makes them excellent areas to study nutrient fate. Previous research has identified karst systems as being unable to remove pollutants and simply acting as a highway for pollutants (Ford and Williams 1989). Within the Floridan Aquifer system this may not be entirely true, Heffernan et al. (2012) identified that substantial denitrification is occurring in these aquifers, effectively removing pollutant nitrogen from the water column. One issue raised by this study is the extreme variation in denitrification values for springs with very low end levels of dissolved oxygen, which this paper will attempt to address in terms of varying landuses.

Objective

The objective of this paper is to test multiple hypotheses regarding aquifer denitrification. (1) Varying landuses manipulate and influence biogeochemical cycling. Therefore, there should be a reflection of nitrogen loading based on varying landuses, with landuses known to have higher nitrogen export (i.e., cultivated crops, pasture land, urban areas) being reflected at the point of measurement. (2) The amount of nitrogen entering an aquifer system is related to the amount of denitrification. Thus, varying concentrations of nitrogen inputs from watersheds with assorted landuses should influence rates of denitrification. (3) Within aquifers, denitrification is show to select lighter isotopes of nitrogen for removal. The isotopic signatures of nitrogen from cropland fertilizers, municipalities, and septic systems are unique from those found naturally (Kreitler 1979, Flipse and Bonner 1985, Aravena et al. 1993, Wassenaar 1995, Panno et al. 2001, Cole et al. 2006). All else being equal, the proportion of this nitrogen should accumulate in aquifers with municipalities and cropland in the watershed. (4) Denitrification occurs under reducing conditions, being more prevalent under hypoxic and anoxic conditions. Geological structures that impact the confluence between the surface and groundwater should impact the rate at which nitrogen is removed from an aquifer.

Methods

This project utilizes USGS landuse/land cover data, Florida Aquifer Vulnerability data, previously collected geospatially referenced (spring location) aquifer denitrification rates, NO_3^- remaining, and calculated values for source $\delta^{15}N\text{-}NO_3^-$. In areas where no existing digital watersheds file exists, ESRI

ArcGIS along with a 10 meter Digital Elevation Model (DEM) was used to delineate the watersheds. Regression analysis was used to determine the influence of the landuse and the water quality parameters on denitrification. The water quality data was also regressed against the landuse and land classification data.

Site Selection

The springs for this study were chosen from the previous Heffernan et al 2012 study and are all located within the Upper Floridan Aquifer System in the middle to upper parts of Florida. In this study, spring locations were chosen based on a review of previous works where information was known about the springshed area, as well as through an extrapolation of springsheds based off their rates of discharge. For more information, see the Springshed section under *Spatial Layers* below. Data for total nitrogen, excess nitrogen, and total dissolved soils were collected for these locations. This information is used in this analysis to determine the influence of these parameters.

Data Collection

Water Quality Point Measurements

The original point measurements used in this analysis included nitrate, discharge, dissolved oxygen, along with a computed excess entrained N_2 gas that can be inferred to be from denitrification (Heffernan et al. 2012). The nitrate data was combined with the excess N_2 gas measurements to obtain a value for total nitrogen. This measurement is a nitrogen flux and should not be confused with total Kjeldahl nitrogen. Additionally, data collected in the Heffernan et al. study on isotopic fractionation of Nitrogen was used to determine source contribution.

Waste Water Treatment Plant Data

The Wastewater Treatment Plant (WWTP) data was provided by Grant Weinkam of UF Water Institute, which consisted of all the domestic EPA registered WTP facilities in Florida from the Florida Department of the Environment (FDEP) as well as values for wastewater reuse and effluent discharge by facility. This dataset was subset by counties where the study springs were located. Data on water land application (reuse) and general discharge (effluent) were used to determine how much wastewater was input into each springshed. Measurements from multiple years were averaged and all averages are given in million gallons per day (MGD).

Spatial Layers

Springsheds

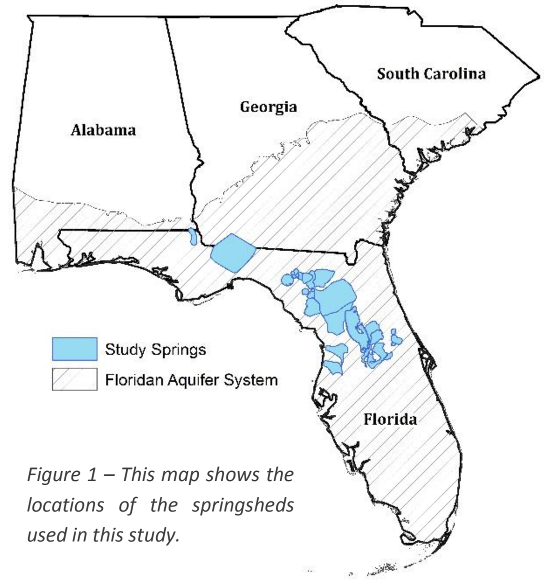


Figure 1 – This map shows the locations of the springsheds used in this study.

Springsheds are the representative watershed for a spring. They are in affect the groundwater contributing area that leads to a springs flow. The springsheds in this analysis were either adopted from previous work by Heffernan et al. (2012), or created using their established methodology of applying the ratio of springshed area to discharge from previously gathered values and applying that to create the simplest springshed. Most of the springshed layer data was provided by Matthew Cohen, Ph.D. This same methodology was applied to 11 springsheds that were recreated for this study (Appendix 1). The discharge data used along with the scaling factor was obtained from Bulletin 66. To create these layers, a 30m DEM from the United States Geologic Survey (USGS) was analyzed to determine the uphill area from a spring so as to correctly orient the springshed. USGS National Hydrography Dataset (NHD) was used to modify the springsheds so they do not cross major bodies of water in concordance with the methodology outlined in Heffernan et al. (2012). Ruth and Palms springs were omitted from this analysis due to missing flow data indicating that these springs may no longer be flowing or their springsheds may be greatly altered.

National Landcover Data

The 2006 USGS National Land Cover 30m grid data was used for this analysis. These layers were created using unsupervised classification of Landsat Enhanced Thematic Mapper+ (Fry et al. 2011). These data were used to determine the amount of each landuse/land cover coverage by springshed. This data was reported in cell counts.

These landuse land cover (LULC) data were binned by complementary classes to reduce autocorrelation and confounding landuse impacts (i.e., emergent herbaceous wetlands and woody wetlands on a springshed scale likely do not have a different signatures in the aquifer). Furthermore, all LULC's that contributed less than about 1% of the total coverage of the springsheds or the total coverage for the study were placed into a single bin of "Other" including: Barren Land (Rock/Sand/Clay); Developed, Medium Intensity and Developed, High Intensity. The full list of landuse classes used and their respective abbreviations are as follows: Barren Land (BL); Cultivated Crops (CC); Deciduous Forest (DF); Developed, High Intensity (DHI); Developed, Low Intensity (DLI); Developed, Medium Intensity (DMI); Developed, Open Space (DOS); Emergent Herbaceous Wetlands (EHW); Evergreen Forest (EF); Grassland Herbaceous (GH); Mixed Forest (MF); Open Water (OW); Pasture/Hay (PH); Shrub/Scrub (SS); Woody Wetlands (WW); Grass and Pasture Lands (GPL); Total Forest (TF); Total Wetlands (TW); and Other (OTH). These binned classes along with all of the classes include in the OTH classification were then extracted by location using springsheds as the bounds for these locations. The descriptions of these layers can be seen in Appendix 5.

Florida Aquifer Vulnerability Index

The Florida Aquifer Vulnerability (FAVA) index layers were used to determine the susceptibility of the springsheds according to the methodology outlined in the FAVA publication by the Division of Resource Assessment and Management of the Florida Geological Survey (Arthur et al. 2005). The most sensitive FAVA layer area in terms of contamination, 'more vulnerable', was extracted. The 'vulnerable' layer was excluded due to autocorrelation with the 'more vulnerable' layer, which is more representative of the aquifers true vulnerability.

Two springsheds, Jackson Blue and Wakulla, were omitted for the analysis of FAVA as they crossed over into Alabama and Georgia respectively and the FAVA layers are only for Florida. The use of the DRASTIC susceptibility index was considered for use, but further review noted that it is an inaccurate representation of the potential risk. DRASTIC places too much weight on elevation and not enough emphasis on the role of karst (Arthur et al. 2005).

Wastewater Treatment Plants

WWTPs were geocoded to their respective location in Florida using ESRI World Geocoding Service. When this service returned multiple locations the most similar address to the input address was selected. The one exception to this was the Miller Street WWTF whose address was acquired though a simple web search and the location given by the ESRI was corrected accordingly.

The spatial relationship of the WWTPs to springsheds was determined by using the 'select by location' tool to spatially join the geocoded WWTPs by the springshed shapefile with a 30 meter buffer. This buffer is based off the digital elevation cell size used in the development of the springsheds. All average MGD values for a springshed were summed together to get a water reuse or effluent value for the springshed in which they are located. These relationships were used to make inferences on nitrogen loading, denitrification, and $\delta^{15}N\text{-}NO_3^-$ concentrations. Total discharge was used as a proxy for nitrogen loading from WWTPs. This loading value was then compared against observed total nitrogen and denitrification as well as source NO3 ($\delta^{15}N\text{-}NO_3^-$) values to determine any significant correlations.

Data Analysis

The data was analyzed using R Statistical Package (R Core Team 2013). Basic statistics were computed for the relevant landuse classes as well as for the water quality parameters and WWTP values. A linear model was used to test trends in the data. If the simplest model's residuals failed the Shapiro–Wilk's test ($\alpha = 0.05$) for normality, then the estimates were determined by bootstrapping. This was accomplished using "An R Companion to Applied Regression" package (car) which utilizes the "boot: Bootstrap R (S-Plus) Functions" package (boot) (Fox and Weisberg 2011, Canty and Ripley 2013) in the R software.

Water Quality Parameters and Connectivity of Aquifers

Dissolved oxygen and total nitrogen were regressed against denitrification to determine a value for the relationship they have with denitrification in the dataset. Vulnerability was regressed against dissolved oxygen, denitrification, and total nitrogen to determine any role connectivity may play in these relationships. Furthermore, a simple regression of source NO_3^- (a value for $\delta^{15}N\text{-}NO_3^-$) was regressed against denitrification.

Comparison of WWTPs Influence on Spring Nitrogen Levels and Species

The simple linear model approach was used to evaluate the influence of WWTP discharge on springshed nitrogen loading, denitrification, and isotopic composition of the outflow. For each parameter of interest, effluent and reuse, values in MGD were regressed against the corresponding dependent variable.

Regression of Water Quality Parameters by Land classes

Various models were created for the comparison of total nitrogen, dissolved oxygen, and denitrification to landuse classes. Furthermore, the most complex model created for this analysis included all the interaction terms for landuse, dissolved oxygen, and total nitrogen regressed against denitrification. The models were developed so that all parameters would be significant at $\alpha = 0.05$. Multiple models were created and the significance of those models were compared to one another.

There were two approaches to reduce the statistical models. In the first, all non-significant parameters were dropped from subsequent analysis after the first run and all subsequent runs until only significant parameters remained (Method 1), In the second, parameters were reduced piecemeal by removing the parameter with the highest p-value until only significant parameters remained (Method 2). Method 2 is intentionally conservative so as to provide an alternative analysis to that of the highly reduced model created by Method 1.

Both models were applied to the full list of landuse land classes and also to the binned landuse classes approach mentioned in the National Land cover Data section. If any of the model residuals were determined to be non-normal, bootstrapping was utilized. To further eliminate any non-significant parameters a comparison was done on the 95% confidence intervals created for said parameters. If this interval spanned 0 then the parameter was removed in concordance with the appropriate methodology. The simplified bootstrapped model's residuals were again tested for normality and this process was continued until all the parameters were found to be statistically different from 0.

During analysis of the Binned Classes, if the Other class was found to be significant it was split into its component parts: Developed, Medium Intensity (DMI); Developed, High Intensity (DHI); and Barren Land - Rock/Sand/Clay (BL) to determine which, if any, of the factors were deemed statistically significant. The previous methodologies were applied to the regression with the parsed out Other term.

Results

Initial Findings

Initial Correlation plots did not show many strong relationships in the data. The exceptions were the obvious strong relationships between total Nitrogen and Denitrification (0.725), Denitrification and Dissolved Oxygen (-0.619), as well as total Nitrogen and Nitrate remaining (0.758). Furthermore, there were autocorrelations in the urban landuses. A correlogram of the data can be seen in the Appendix (Appendix 4).

Springsheds

Of the 24 Springsheds used in this study, Santa Fe accounted for about ~18% of all the 111 observations. Furthermore, fours springs (Santa Fe, Ichetucknee, Silver, and Silver Glen) represent over half of the study observations. The areas of the springsheds used for analysis are calculated by converting the LULC raster cells used in this analysis to approximate their area. Total area was seen to be skewed to the right (skewness= 0.9298) with a median of 991.8 km^2. The total landcover areas by point measurements and by springsheds were not statistically different ($\alpha = .01$) see Table 1 in the Appendix).

Wastewater Treatment Plants

The geocoding program run in ESRI ArcMap returned 15 results that had tied address values. These addresses were corrected according to the methods outlined in the previous section. Of the 25 springsheds from the study, 15 had no registered WWTPs within their borders, Jackson Blue and Wakulla were also omitted from this analysis. Three springsheds that contained WWTPs had no data for discharge from the period of 2007 – 2010 and therefore were omitted from analysis leaving Alexander, Gemini, Green Spring, Santa Fe, Silver, and Wekiwa. Alexander and Green Spring were also omitted for the source NO3 analysis due to a lack of data.

Water Quality Parameters and Connectivity

All of the water quality parameters were skewed to the right. NO_3^- had a median of 1.04 mg/L and Total N has a median of 1.720 mg/L. Denitrification was shown to have a median of 0.664 mg/L. Dissolved Oxygen had a median 2.005 mg/L and a min of 0.010 mg/L (DL), with a maximum of 7.360 mg/L. A histogram of these parameters can be seen in Appendix 3. The isotopic signature was shown to have a minimum value of -0.85 ($\delta^{15}N\text{-}NO_3^-$) and a maximum 18.3, with a median of 4.2.

All but the three springsheds omitted from the vulnerability analysis were considered at least partly vulnerable according to FAVA index. The minimum value for vulnerable springs was over 50% and the maximum value was 100% coverage of the most vulnerable layer. The median vulnerability was 89% and indicated the strong right-skewness of the data.

Landuse/Land Classification

Landuse for the springsheds is dominated by Forest (~30%) with Evergreen Forest compiling the majority of those measurements. In fact, of the 111 observations 79 had Evergreen Forest as their dominant landuse. The second most abundant landuse types are Grass and Pasture and Wetlands (~20%) with the springshed subset having more Wetlands and the full data having more Grass and Pasture (see the significant difference in the subsequent section). These three classes account for about two-thirds of all the land cover data when subset by springshed.

With regards to dominant landuse for each springshed, Evergreen Forest was the mode. The second most dominant landuse types by point measurements was Developed, Open Space, followed by Pasture/Hay, Woody Wetlands, and Shrub/Scrub. When the landuse classes were binned together Total Forest, driven by Evergreen Forest, was once again the most dominant landuse with the same springs falling out of the analysis. Grass and Pasture Land was the second most dominant land cover for the springs (~23%). With Developed, Open Space and Total Wetlands being the third and fourth most populous land covers.

Data Analysis

Denitrification regressed by the total wastewater effluent was found to be statically significant (p-value = 0.000121). The influence of measured effluent was positive and highly significant, explaining ~27% of the variation in the data. When the reuse values were regressed against denitrification the correlation was once again positive, but this regression explained about ~43% of the variation in the data and was found to be more significant (p-value = 2.569E-07). There was no difference in the magnitude of influence for the effluent or reuse values.

Wastewater Treatment Plants

The regression of denitrification regressed by the total wastewater effluent was found to be statically significant (p-value = 0.000121). The influence of measured effluent was positive and highly significant, explaining ~27% of the variation in the data. When the reuse values were regressed against denitrification the correlations was once again positive, but this regression explained about ~43% of the variation in the data and was found to be more signification (p-value = 2.569E-07). There was no difference in the magnitude of influence for the effluent or reuse values.

For the regression of total nitrogen regressed by WWTP effluent the residuals of the initial linear model was not normally disturbed and therefore a bootstrap analysis was conducted. This analysis was similar to that of the regression for denitrification values, but was found to explain slightly more of the variability (Adj R-sq = 0.3464). Once again the reuse value showed a stronger relationship explaining ~57% of the variation in the data. Furthermore, reuse showed an order of magnitude more influence than that of the total nitrogen regressed against wastewater effluent (β = 1.10E-01 and β = 8.93E-02 respectively).

The regression for source $\delta^{15}N\text{-}NO_3^-$ was not significant for either effluent or reuse, possibly due to the extremely small sample size.

T Test for Between Landuse Groups

Landuse classes showed no significant difference at *α = .01*, with the exception of GPL showing a slightly significant difference. GPL was barely significant (p-value = 0.009391) when evaluated at this critical value (see Appendix 2). When GH and PH were unbundled there was no apparent significant difference between GH and the significance found for GPL, indicating that PH may be driving the significance of GPL (student-t = 2.687, p-value = 0.008).

Water Quality Parameters

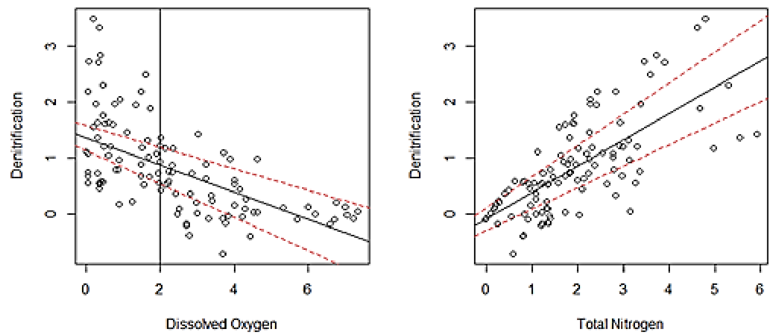


Figure 2 – The above graphs show the data for DNF by the water quality parameter for which they were regressed against. The associated regression lines are also shown as well as their 95% confidence bands in red. For the dissolved oxygen graph a vertical line is shown for the threshold of creation for nitrate reductase which is necessary to reduce nitrogen back to its elemental form (Körner and Zumft 1989).

When dissolved oxygen was regressed against denitrification it was found to have a negative relationship and explained about 38% of the variability in the data (β = -0.24451; Mx R-sq = 0.3858; Adj R-sq = 0.3801).

Total nitrogen regressed against denitrification was found to have a positive relationship and explained about 50% of the variability in the data (β = 0.46941; Mx R-sq = 0.5095; Adj R-sq = 0.5049).

Vulnerability - FAVA

A regression analysis of dissolved oxygen regressed by vulnerability reiterates the positive relationship shown in the correlogram. This relationship was found to be significant at $\alpha = 0.05$. The regression analysis of total nitrogen regressed by vulnerability also showed a positive relationship and was a bit stronger (p-value = 0.00684) than that of the relationship between dissolved oxygen and vulnerability. Both models did a poor job of explaining the variability in the data.

When vulnerability was regressed against denitrification no significant relationship was identified, but when denitrification was regressed by vulnerability and dissolved oxygen with the interaction of the two the relationship was significant. The significant parameters were vulnerability and the interaction of vulnerability and dissolved oxygen. This significance persisted after the dissolved oxygen term was removed. Vulnerability was shown to have a positive influence on denitrification and the interaction of vulnerability and dissolved oxygen had a negative influence.

Regression Analysis of Landuse Influences

Denitrification

Original Land Classes – Method 1

For the original land classes model BL, EHW, and WW were all found to be significant (Mx R-sq = 0.2177; Adj R-sq = 0.2177). BL and WW were found to have a positive influence on denitrification with BL having an order of magnitude greater influence (β = 6.28E-05 and β = 3.86E-06 respectively). EHW was seen to have a negative influence on denitrification and was also an order of magnitude stronger in effect than that of its woody counterpart (β = 6.28E-05). As for BL and EHW, they had a statistically similar difference in influence although in the opposite direction.

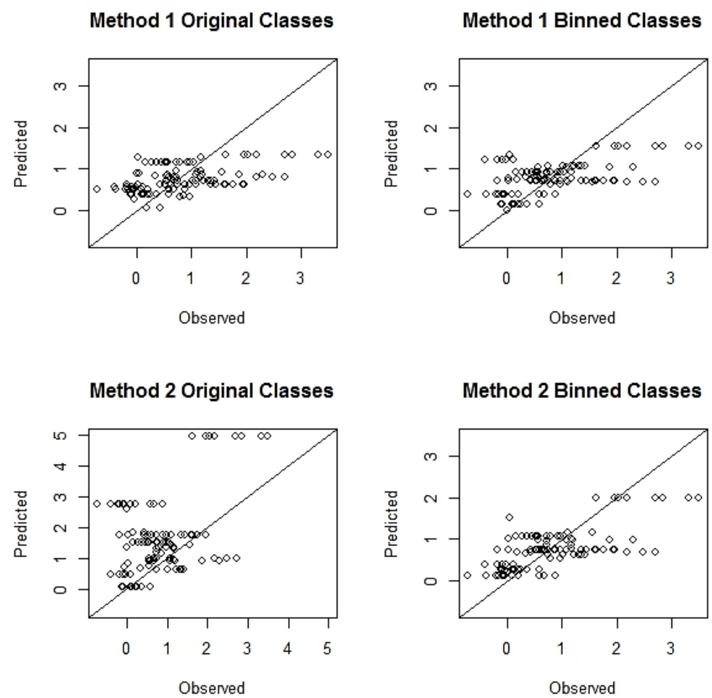


Figure 3 – Above are graphs of the observed vs predicted values with the 1:1 line shown for the regression of denitrification by LULC. Note the spread for method one. Also note that Method 2 with binned classes seems to be more clustered then all other observations. Furthermore, all graphs have the same minimum (0.061 mg/L), and all but Method 2 Original Classes have the same maximum (1.33 mg/L) values from the observed data. Method 2 Original Classes' maximum was 4.973

Binned Land Classes – Method 1

The Binned Land Classes explained slightly more of the variation in the data (Mx R-sq = 0.2876; Adj R-sq = 0.2672). CC and SS showed a statistically similar positive influence on denitrification (β = 5.84-06 and β = 2.43E-06 respectively). TF showed a negative influence (β = -1.94E-06) and was statistically similar in its weight of influence to that of CC and SS.

Original Land Classes - Method 2

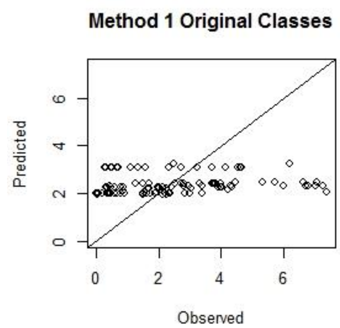
Method 2 showed an increase in explanatory power (Mx R-sq = 0.4862; Adj R-sq = 0.4506) as compared to method 1. BL, DHI, EF, PH, SS, and WW were found to be significant parameters. BL, SS, and WW all had a positive influence on denitrification with BL (β = 2.64E-04) showing the greatest impact. SS showed a slightly increased influence compared to that of WW (β = 1.46E-05 and β = 5.10E-06 respectively). PH, EF, and DHI had a negative influence on denitrification. PH and EF did not differ statistically from one another but both had statistically less of an influence than that of DHI (β = -2.32E-04).

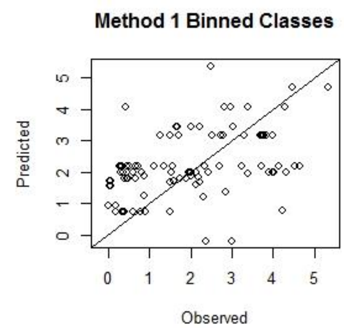
Binned Land Classes – Method 2

Once again the model for method 2 explained more of the variation in the data, but in this instance the un-binned classes for method 2 seemed to be a bit more explanatory (Mx R-sq = 0.4404; Adj R-sq = 0.4132). The statistically significant parameters for this analysis were CC, DLI, GPL, SS, and TF. CC, DLI, and SS were all found to have a positive influence on denitrification with a statistically similar amount of influence for each parameter. GPL showed a slightly stronger negative influence than that observed for TF, although the two were not statistically different as their 95% CI overlapped slightly (-6.49E-06 – -2.72E-06 for DLI and -2.92E-06 – -1.49E-06 for TF).

Dissolved Oxygen

Original Land Classes - Method 1





For this method the only significant parameter was that of EF (α = 0.05). EF was shown to have a positive influence on dissolved oxygen (β = 9.80E-07). This regression explained only about 3% of variability in the data (Mx R-sq = 0.04332; Adj R-sq = 0.03446).

Binned Land Classes - Method 1

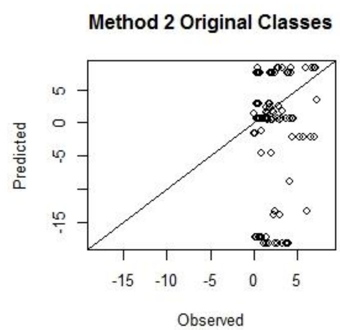


Figure 4 – These graphs depict the observed vs predicted values with the 1:1 line shown for the regression of dissolved oxygen by LULC. Note at the clustering for Method 1 Binned Classes, this was also the regression that resulted from Method 2 with the binned classes. The irregular intercept for Method 2 Original Classes has to do with predicted values that were well below zero with dissolved oxygen values of less than -18 mg/L predicted.

The binned model seemed to explain about a third of the variability in the data (Mx R-sq = 0.3414; Adj R-sq = 0.3163) with GPL, SS, TF, and TW being the significant parameters. This model was highly significant as compared to that of the original land classes model (p-value = 5.69E-09). GLP and TF showed a positive influence on dissolved oxygen levels and SS and TW showed negative levels. All the variations in the parameters were not statistically different from one another when sign changes were ignored.

Original Land Classes - Method 2

This method developed a model that had much more explanatory power than the previous method both in terms of model significance (p-value = 1.86E-10) and in terms of its ability to capture the variability in the data (Mx R-sq = 0.4498; Adj R-sq = 0.4062). The significant parameters were BL, DHI, DMI, EF, PH, SS, and WW. Of these parameters BL and DMI had a slightly similar negative influence on denitrification with their 95% CI crossing slightly (-1.14E-03 – -4.65E-04 respectively -4.79E-04 – -1.96E-04). PH and WW were statistically similar in their negative influence on denitrification and were an order of magnitude less influential. Of the landuses with positive influence, EF showed the least effect (β = 1.93E-05). SS showed a similar level of influence (β = 4.69E-05), but was statistically different from that of EF. DHI was 2 orders of magnitude more influential (β = 1.67E-03).

Binned Land Classes- Method 2

The binned regression was the same as the binned regression created by method 1.

Total Nitrogen

Original Land Classes - Method 1

Only CC and OW were found to be slightly significant (α = 0.05). CC was shown to have a positive influence on total nitrogen and OW was seen to be negative (β = 4.72E-06 and β = -7.44E-06 respectively). This regression explained only about 6% of variability in the data (Mx R-sq = 0.06493; Adj R-sq = 0.02664) and 5% when corrected for the number of parameters. The model itself was only loosely significant at a critical value of 0.05 (p-value = 0.02664).

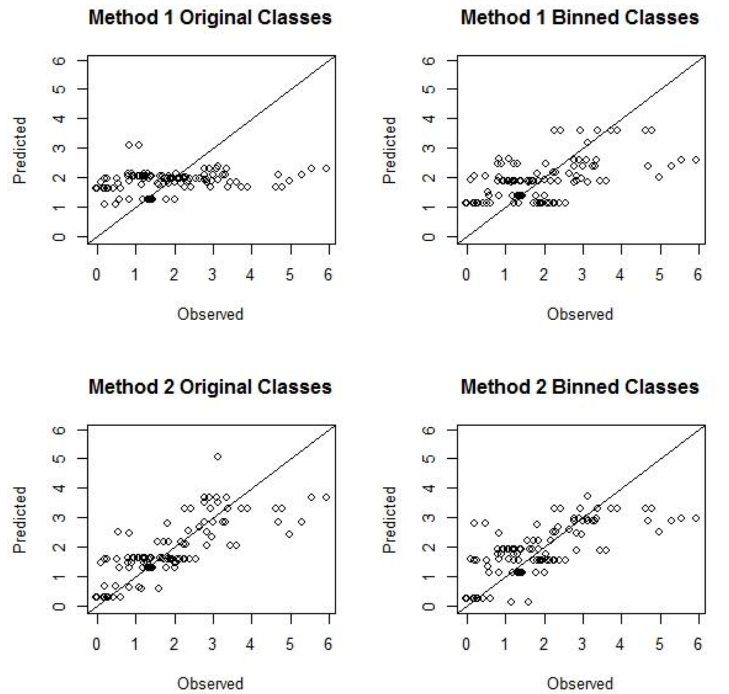


Figure 5 – Above are graphs of the observed vs predicted values with the 1:1 line shown for the regression of total nitrogen by LULC. Note how method one shows only a slight relationship as compared to the method 2 models. This binned classes shows a strong correlation, but when the Other class was parsed out into its component terms the regression was the same as that of method 1s binned classes.

Binned Land Classes - Method 1

CC and TF were found to be significant, with CC having a positive influence (β = 1.25E-05) on total nitrogen and TF shown to be negative (β = -7.44E-06). This model had more explanatory power than that of the previous method, explaining ~30% of the variability in the data (Mx R-sq = 0.3013; Adj R-sq = 0.2883). The influence of CC was stronger than that of TF.

Original Land Classes - Method 2

This model explained nearly 60% of the variation in the data with CC, DF, DLI, DMI, EF, OW, PH, and WW all found to be significant parameters (Mx R-sq = 0.6005; Adj R-sq = 0.5692). DMI, CC, and WW all had a positive influence on nitrogen levels with DMI's influence greater than that of CC (β = 1.35E-04 and β = 3.10E-05 respectively). WW showed the least amount of positive influence (β = 1.23E-05). On the other end DF and DLI showed similar negative influence on nitrogen levels about on par with the positive influence of DMI (95% CI -1.88E-04 – -7.89E-05 DF; -8.70E-05 – -3.84E-05 DMI). DMI's influence was also similar to that of WW's positive influence. OW, EF, and PH did not have statistically different negative influences, and the most amount of variation was that of OW (95% CI -3.17E-05 – -6.62E-07).

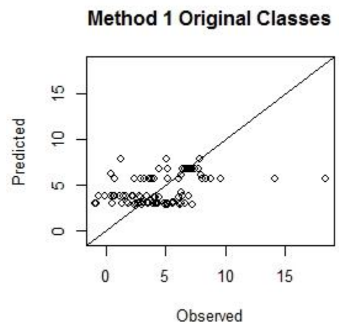
Binned Land Classes - Method 2

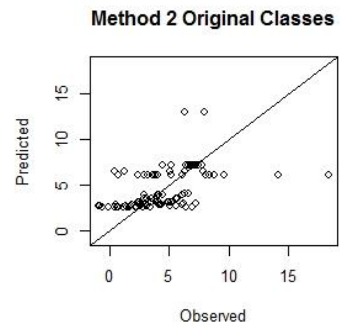
Initially CC, DLI, DOS, SS, TF, TW, and Other were found to have statistical significance with the modeling being slightly less explanatory than that of the original land classes (Mx R-sq = 0.4884; Adj R-sq = 0.4536). DLI and DOS were shown to have similar negative influences on nitrogen as well as DOS and TF (95% CI -5.49E-05 – -1.22E-05 DLI; -2.87E-05 – -3.69E-06 DOS; -9.93E-06 – -6.03E-06 TF). CC, SS, and TW all showed a similar positive influence on nitrogen levels. The Other term was significantly higher than all other positive influences, but when the Other term was parsed out into its component parts the binned regression was the same as the binned regression created by method 1. This influence was not greater than that of the negative influence observed for DLI.

Source Isotopic Nitrogen Signature

Original Land Classes - Method 1

The only significant parameter for source NO_3^- was that of DLI and the explanatory power of the model was very low at ~10% (Mx R-sq = 0.134; Adj R-sq = 0.124). DLI was shown to have a positive influence on source NO3 (β = 3.69E-05).





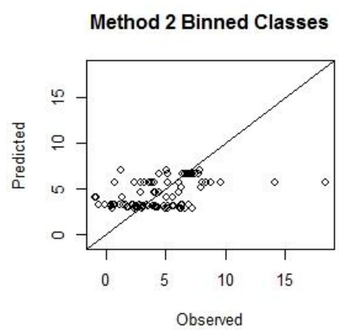


Figure 6 – These graphs depict the observed vs predicted values with the 1:1 line shown for the regression of estimated source $\delta^{15}N\text{-}NO_3^-$ by LULC. Method 2 Original Classes appears to be the most dispersed and Method 2 with Binned Classes is most tingly clustered around the 1:1 line, which presents a different story from that of the adjusted R-sq value for each model.

Binned Land Classes - Method 1

There were no significant parameters found for source NO_3^- regressed by landuse for this method, but WW was found to be significant at 0.1.

Original Land Classes - Method 2

This model had twice as much explanatory power than the previous methods model, but still only explained ~20% of the variability parameters (Mx R-sq = 0.2324; Adj R-sq = 0.2053). PH, SS, and WW were found to be significant with PH and WW having a positive relationship with $\delta^{15}N\text{-}NO_3^-$ and SS was found to have a negative relationship. PH and WW's influences were not statistically different from one another and SS's negative influence was comparable in magnitude (95% CI 4.25E-06 – 1.41E-05 PH; 6.28E-06 – 1.67E-05 WW; -1.92E-05 – -9.14E-06 SS).

Binned Land Classes - Method 2

For the binned classes after bootstrapped parameters were removed (95% CI's that overlapped 0) only OW remained as a significant parameter. This model had similar explanatory power to that of method 1's model (Mx R-sq = 0.1686; Adj R-sq = 0.159). OW was shown to increase $\delta^{15}N\text{-}NO_3^-$ (β = 3.72E-05).

*Full Model – Denitrification ~ Land Classification*Dissolved Oxygen*Vulnerability*

Original Land Classes - Method 2

No significant parameters were found using the first methodology, but for Model 2 with the original land classes was shown to explain about ~50% of the variation in the data (Mx R-sq = 0.5202; Adj R-sq = 0.5065). CC and DF were found to be significant land uses with the interaction of PH and dissolved oxygen also found to be significant. CC was shown to have a positive influence (β = 9.63E-06) on denitrification and DF was shown to have a negative influence (β = -2.63E-05) with an order of magnitude more than that of all the other parameters. The interaction term of PH and dissolved oxygen showed a negative influence and was two orders of magnitude lower in influence (β = 9.63E-06) than that of DF.

Binned Land Classes - Method 2

For the Binned Land Classes model ~70% of the variability in the data was explained (Mx R-sq = 0.7329; Adj R-sq = 0.7078). The landuses CC, DOS, GPL, TF, and DLI (in the term DLI*Dissolved Oxygen) were all found to have significant influence on denitrification. Of these landuses only GPL was found to have a direct positive influence on denitrification.

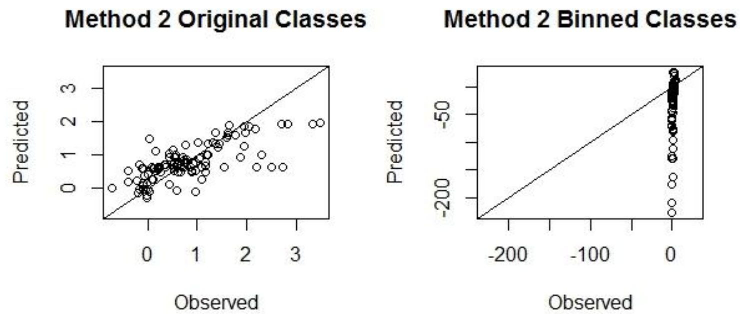


*Figure 7 – Above are graphs of the observed vs predicted values with the 1:1 line shown for Denitrification regressed against LULC*Dissolved Oxygen*Vulnerability. Note how method 2 binned classes has predictions well outside of the observed data and some of these results are also well outside of biological feasible values. Method 2 original classes shows clustering around the 1:1 that is better than that observed by the binned classes, likely due to the simpler terms used in the original classes model. Furthermore, this model shows clustering that is as good as or better than all previous regression of denitrification.*

Dissolved oxygen also fell out as a significant term and as was the case with the previous regression analysis the influence was negative related to denitrification (β = -1.03E-01). Furthermore, dissolved oxygen was shown to have three orders of magnitude more influence than the next closest term. There is also a term for the interaction of dissolved oxygen and DLI that was shown to have a negative relationship (β = -2.12E-06) with denitrification.

Vulnerability was shown to have a significant relationship when interacted with CC, DOS. The most complex term also included the GPL interacted with dissolved oxygen. The interaction terms from Vulnerability and CC as well as Vulnerability and DOS were shown to have a positive relationship with denitrification (β = 1.54E-04 and β = 1.05E-04 respectively). Whereas the term of GPL*Dissolved Oxygen*Vulnerability is shown to have a negative relationship, likely due to the inclusion of dissolved oxygen. This model contains many interaction terms and therefore caution should be taken when drawing any conclusions on the relationship that this model presents. Furthermore, predictions by this model are well outside of realistic values.

Discussion

Factors Influencing Nitrogen Cycling in Floridan Springs

Across the study landscape there are major sources of variation in nitrogen cycling that are attributable to a variety of different topics. One of these is the relevance of nitrogen loading on rates of denitrification. In general, the data showed that as nitrogen loading increased denitrification also increased. Additionally, the data showed a lot of variability in dissolved oxygen concentrations, which had a significant relationship with denitrification. This relationship is expected as dissolved oxygen is a constraining factor for denitrification.

There was abundant variability in dissolved oxygen levels and corresponding denitrification. Jackson Blue is observed to have low levels of denitrification and is well outside the dissolved oxygen threshold for denitrification. This could be due to a natural process by which microbes can create anaerobic micro environments facilitating denitrification, a process observed in karst systems of Franconia Germany (Seiler and Vomberg 2004). This process may be even more likely for the Rainbow springshed, which has very low nitrogen loading with a comparable median denitrification to that of the Jackson Blue springshed. Although, it is possible that this relationship may just be a reflection of the observed dissolved oxygen concentrations being closer to that of the threshold for denitrification.

The status of aquifer vulnerability in nitrogen cycling appears to be expressed through impacting nitrogen loading and dissolved oxygen levels, but not through any significant direct relationship with denitrification. The relationship of vulnerability to dissolved oxygen is positive, which indicates that as connectivity of the surface to the aquifer increases so does the levels of dissolved oxygen observed an the output springs. Vulnerability relationship in karst systems is due to the creation of preferential flowpaths into aquifers, which in turn alter the chemistry of the aquifer below (Tihansky and Knochenmus 2001). These flowpaths likely increased gas exchange associated with the connection of the aquifer to the atmosphere.

Human Wastewater and the Aquifer Nitrogen Cycle

It appears that WWTP outflows may have an influence on how nitrogen is being cycled in this system. As previously stated by Katz et al (2009), nitrate derived from WWTPs is observed downgradient of sprayfields in Wakulla Springs. This relationship was once again observed in this data likely indicating that this may be a more generalizable trend.

Furthermore, when wastewater is land applied instead of injected into deep wells or discharged into a stream the influence of the nitrogen in the spring is more pronounced. The process of land application, although intended to allow for ample time for nitrogen to return to its atmospheric pool, often is not wholly effective (Katz et al. 2009). As for the injection wells, it is possible that over time the influence of wastewater injected into wells may become more pronounced as the slow exchange between the different aquifers allows for the transport over longer time scales than that of the surface to the upper aquifer.

All these findings should be taken with a bit of reservation due to the lack of statistical power. With a sample size of only 5 truly unique measurements this power drops to ~17% for the relationship of denitrification regressed by effluent and ~22% for total nitrogen regressed by effluent. The power for the water reuse regression is slightly better at ~29% for denitrification and ~42% for nitrogen loading.

What Role Landuse has to play in Aquifer Nitrogen Cycling

All inferences made from this analysis should take into account that there were few dominant land types and that in total all the observed area was predominantly evergreen forest. Furthermore, Pasture/Hay was sampled at a different rate when considering the multitude of point measurements. That being said it may also strengthen the perceived effect of the less dominant land types adding credence to the importance of their effects when it outweighs the main landuses.

Open Water

Open water was identified to have a significant relationship with nitrogen loading showing a strong negative correlation. This open water is often flowing and if there is not much hyporheic exchange than the nitrate found in these waters would be easily exported from the system never reaching the aquifer below. It is also possible that efficient use of nitrogen in the surface waters by algae may be absorbing and mineralizing what would be a more mobile form of nitrogen. This is even more probable in climates with high solar inputs and warm temperatures.

Previous studied have identified that there is a negative relationship with human impacts on surface water and the ability of dissolved oxygen to be transported to the groundwater. This relationship is thought to be based off the blocking of channels for exchange though the hyporheic zone (Hancock 2002). Interestingly, in this study open water was shown to have a general positive relationship to dissolved oxygen levels (see figure 8), although this relationship was not statistically significant. It is possible this may be due to enhanced oxygenation of the water through increased plant activity due to higher levels of available nutrients. Although, this relationship would switch if there were instances of algal blooms which are often followed by low dissolved oxygen levels as these blooms decay.

Sparsely Vegetated Land

Barren Land was considered a significant parameter in the two denitrification models in which it was its own term, but both models had low explanatory power. However, a significant negative relationship with dissolved oxygen and Barren Land was identified. This relationship is likely the influencing factor in the observed denitrification results for Barren Land.

Shrub/Scrub land was also identified as a significant contributor to denitrification. In fact, this land class was identified as being significant in all denitrification models with the exception of the least explanatory model, Method 1 Original Land Classes. This land class was shown to have a positive relationship with denitrification likely reflecting the ease of nitrogen loading in these xeric landscapes. Lack of vegetation coupled with the sandy soils provides an easy means of atmospherically deposited nitrogen to enter the groundwater though flushing from rainstorms. This is reiterated by a negative relationship between $\delta^{15}N\text{-}NO_3^-$ and scrub land. This nitrogen loading was reflected in the initial method 2 regression which included a significant term for the Other classification and also had slightly less explanatory power than method 2's Original Classes model. This model showed a stronger clustering around the observed versus predict 1:1 line.

If these lands are converted to other uses in the future, such as one that has more grasses though irrigation, it is possible that these areas may move from a nitrogen source to a nitrogen sink. Invading grasses absorb much more nitrogen than that of the sparse vegetation and also take much longer to decompose reducing carbon exports, another component necessary for denitrification, not addressed in this study (Wolkovich et al. 2010).

Shrub/Scrub land cover also shows an interesting relationship with dissolved oxygen levels with one of the models showing a positive relationship and the two others showing a negative one. The positive influence on dissolved oxygen levels is likely an artifact of the model, which although having a good R^2 does an extremely poor job of accurately predicting the observed data.

Top 4 Landuses for Total Nitrogen's Relationship to Denitrification

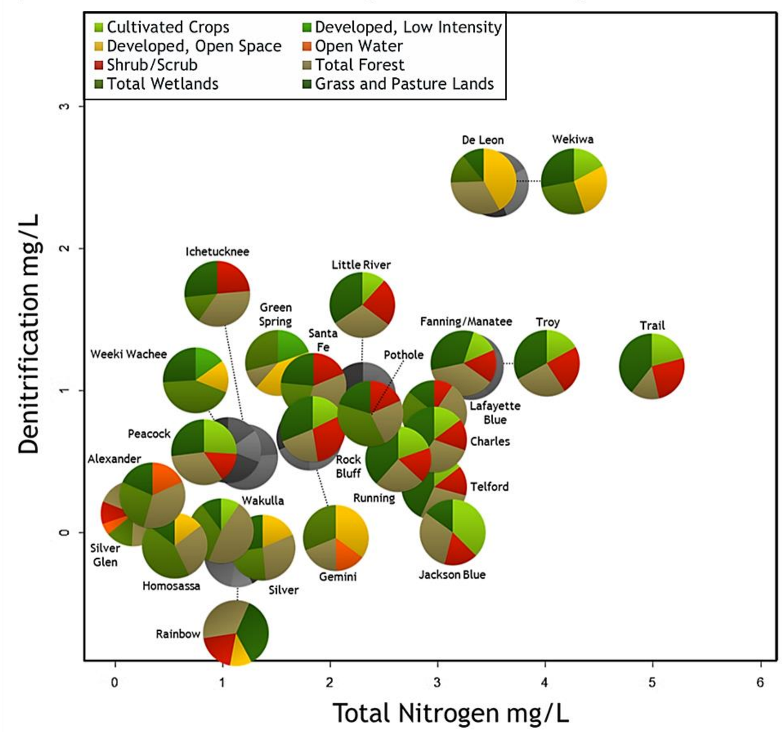
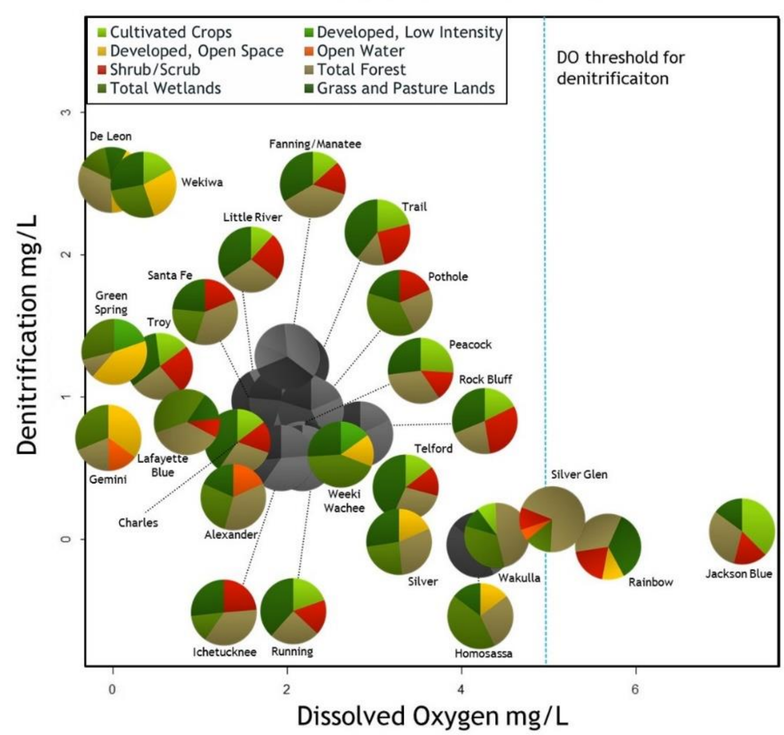


Figure 8 – The graph to the left displays the relationship between total nitrogen and denitrification with a pie chart displaying the top 4 landuses for each springshed plotted in the center of a theoretical box created by the length and width of the interquartile range for the corresponding parameters of total nitrogen and denitrification.. Note how increase Developed, Open Space land cover leads to increasing nitrogen loading and increased denitrification. Furthermore, there is a noticeable decreasing trend in forest cover as you move toward springsheds with more nitrogen loading and resulting increases in denitrification.

Top 4 Landuses for Dissolved Oxygen's Relationship to Denitrification

Figure 9 – The graph to the right displays the relationship between dissolved oxygen and denitrification with a pie chart displaying the top 4 landuses for a springshed plotted in the center of a theoretical box created by the length and width of the interquartile range for the corresponding parameters dissolved oxygen and denitrification. A vertical line is placed at 5 mg/L dissolved oxygen to indicate the threshold for the creation of nitrongnase enzyme (Körner and Zumft 1989)*. This enzyme is a precursor to the enzymes required to fully reduce aqueous nitrogen species to nitrogen gas.*



Cropland

Cultivated Crops were shown to play a major role in this aquifer system, both through evidence of increasing nitrogen concentrations in the springsheds, as well as through a corresponding increase in denitrification. The relationship between agriculture and nitrogen loading is likely due to over application of nitrogen fertilizers, which in turn leads to elevated levels of denitrification. That being said, no significant relationship between the isotopic signatures of this nitrogen was identified by the regression analysis of source $\delta^{15}N\text{-}NO_3^-$. If chemical fertilizers played a major role in this system one would expect to see a relationship between Cultivated Crops land cover and depressed $\delta^{15}N\text{-}NO_3^-$ values associated with the "lighter" nitrogen in these fertilizers (Bateman and Kelly 2007). It is possible that this signal is being lost due to the preferential selection for lighter isotopes, with regards to biologically mediated processes such as denitrification. Through this mechanism it is possible that much of the lighter nitrogen is being removed and therefore is not evident at the spring.

When landuse is regressed against denitrification with the interaction of dissolved oxygen, Cultivated Crops is found to be significant when the other class is parsed out into its component parts. This trend is also evident in figure 9 where a visual analysis shows generally that increasing Cultivated Crop land cover leads to lower levels of dissolved oxygen (these observations were clustered around 2 mg/L) and a resulting increase in denitrification. This relationship between agricultural landuse and depressed dissolved oxygen levels in water systems was also shown in King County, Washington (Milburn 2007).

The most complex model developed for this analysis reiterates the importance of Cultivated Crop's positive influence on denitrification with it being one of only two landuse parameters found to be significant. Although the opposite was true for the regression of the binned land classes, this model predicted extraneous values and therefore it may be appropriate to omit this model from our analysis. The most complicated model developed from this analysis, Cultivated Crops goes from having a positive to a negative correlation with denitrification, but when vulnerability is taken into account this relationship changes to one that is positive. This finding may indicate that agricultural lands by themselves may not be major sources of nitrogen that is ultimately removed, but when are located in areas of high connectivity to the aquifer below this relationship increases the observed denitrification.

Wetlands

An interesting relationship surfaced with regards to Emergent Herbaceous Wetlands and Woody Wetlands and its influence on denitrification. It appears that Emergent Herbaceous Wetlands may actually decrease denitrification whereas Woody Wetlands are shown to have a positive influence. It is possible that this may be an artifact of the data as nitrogen loading and dissolved oxygen are not shown to have any significant relationship with Emergent Herbaceous Wetlands.

Total Wetlands and Woody Wetlands were found to have a positive influence on total nitrogen and this may be what is being reflected in the observed denitrification levels. The difference between Emergent Herbaceous Wetlands and Woody Wetlands is further supported by a worldwide meta-analysis of wetlands which showed a greater nitrogen load than all other wetlands (Jordan et al. 2010). This signal is likely elevated by the negative relationship between Total Wetlands and Woody Wetlands observed for dissolved oxygen. An additional strength of these results is that Total Wetlands and Woody Wetlands nitrogen loading signals came from the most explanatory models with the best predictive accuracy. However, when the Other class was parsed out to its component parts Total Wetlands was no longer found to be significant.

This may indicate that Woody Wetlands are truly the main influence with regards to wetlands, a fact brought up previously in this paragraph regarding nitrogen load. This positive relationship observed for total nitrogen levels is likely due to historical loading of nitrogen in these wetlands. In fact, it has been observed that in the US wetlands absorb ~20% of anthropogenic nitrogen inputs (Jordan et al. 2010).

Historical loading may be moving from a previous sink in particular areas to a potential source. When you look at Woody Wetlands themselves this may not be the cause because increases in Woody Wetland land cover showed an increase in the amount of $\delta^{15}N\text{-}NO_3^-$ at the spring. This increase likely reflects the fact that wetlands are a major biological hub and nitrate is being mobilized and mineralized multiple times, leading to a high fraction of this heavier nitrogen.

Urban Areas

Urban areas seem to play a limited role in denitrification in this system. The autocorrelation of urban areas is indicative of the arbitrary threshold placed between the urban classes as well as the spatial relationship that these areas have with one another.

Areas that are classified as Developed, High Intensity appear to have decreased levels of denitrification, and a corresponding increase in dissolved oxygen content. The relationship identified for dissolved oxygen was strong and two orders of magnitude more influential than all other parameters in the model. It is possible that this signature reflects the higher concentrations of dissolved oxygen in rainfall that are being piped (both directly and thought infiltration fields) into the aquifers though stormwater systems.

The Developed, Medium Intensity class was found to be significant and negatively correlated with dissolved oxygen levels. This correlation may be an inaccurate representation as many of the predicted values are well outside of a realistic range. In fact some of these values were as low as -18 mg/L. The relationship of medium intensity development and total nitrogen was shown to be positive, which may be a reflection of more people with lawns that are being managed in these areas as compared to developed, Low Intensity. These lawns can be a source of nitrogen pollution in groundwater.

Developed, Low Intensity, unlike that of high intensity developed, displayed a positive influence on denitrification. Oddly enough there appears to be no relationship with dissolved oxygen and a negative relationship with nitrogen loading. This relationship is strong as it was evident in both the binned and original class models for the second method, both of which had high R^2 values (Adj R-sq = 0.4536 and Adj R-sq 0.5692, respectively). Furthermore the nitrogen attributable to this landuse does not appear to be atmosphere derived. This indicates that this nitrogen may actually be attributable to wastewater treatment plants effluent.

Developed, Low Intensity was also significant when interacted with dissolved oxygen in the most complex model, indicating that there may be a relationship between DO and Developed, Low Intensity not identified in the regression of landuse against dissolved oxygen concentrations. This may be the way in which this land classification influences denitrification though changes in dissolved oxygen concentrations.

Increasing amounts of Developed, Open Space land cover have shown an increasing relationship with nitrogen loading and dissolved oxygen, although this relationship was not found to be significant after the Other class was parsed out into its component parts. It is possible that this increasing relationship is due to the multitude of golf courses located in the study area. Nitrogen leeching has been identified to occur from golf courses as well as agricultural lands, through an analysis of isotopic fractionation of N (Flipse and Bonner 1985). That being said, previous studies have shown that nitrogen leeching from golf courses may be minimal, but has the chance to be a major source of contamination if best management practices are not implemented (Branham et al. 1995). Best management practices may not play a major role in determining nitrogen loading to aquifers due to surficial connectivity and preferential flowpaths discussed earlier. This may be the reason that the same relationship in the most complex model for the binned land classes is observed for Developed, Opens Space as was observed for Cultivated Crops.

Forests

Forests played a major role in this analysis and followed typical patterns of low nitrogen export. In fact, in all regressions forest land was shown to have a negative influence on nitrogen loading whether it be Evergreen Forest, Deciduous Forest, or Total Forest. This is to be expected due to lower inputs of anthropogenic nitrogen sources, and the ability of a forest to efficiently use nitrogen (Vitousek 1982). This is partially due to the increased rooting depth of trees as compared to other plant species, and the corresponding increased access to nitrogen throughout the soil column.

Interestingly, there is a positive relationship for forest and dissolved oxygen that appeared in all of the regressions of dissolved oxygen by land classification. This same relationship of dissolved oxygen concentrations being greater in forested watershed, as compared to urban or agricultural lands, was observed in a comparison of three streams (Lenat and Crawford 1994). These two factors are the leading reasons why forested land shows a negative relationship to denitrification in these springs. In fact, this relationship was identified in all but the least explanatory denitrification models. This includes that of the most complex model that takes into account dissolved oxygen, landuse, and aquifer vulnerability. The only other directly identified parameter was that of Cultivated Crops. Another noteworthy relationship is that of the negative influence of forestland on $\delta^{15}N\text{-}NO_3^-$ this corresponds to earlier studies, which identify a lighter signature of nitrogen from forested lands as compared to those of wastewater and slightly heavier than that of fertilizer which is wholly atmospherically derived (Spalding et al. 1982, Aravena et al. 1993, Panno et al. 2001, Petitta et al. 2009).

Grasslands

Both Pasture/Hay landuse and the combined Grass and Pasture Land classification were found to have a negative influence on rates of denitrification. One major reason this likely exists is the cattle grazing as a means of nitrogen reduction, leading to a reduced nitrogen load available for denitrification. This is opposite to findings of a previous study of agricultural lands, which were dominated by pasture land and its influence on nitrogen loading. A regression analysis by Boyer and Pasquarell (1995) identified that for every % increase in agricultural land there was a corresponding increase of 0.19 mg/L nitrate. This study may actually be an indication of the cultivated crops influence on nitrogen loading which is being buried in the clumping of the two land uses. Even with no direct relationship of nitrogen and grasslands, it is likely that some of this nitrogen is entering the aquifer as evidenced by the positive correlation of Pasture/Hay lands and $\delta^{15}N\text{-}NO_3^-$. Heavier nitrogen is indicative of waste products which are likely to accumulate on pasture lands (Spalding et al. 1982).

There were mixed results as to grasslands role in dissolved oxygen concentrations, with Pasture/Hay showing a negative relationship and Grass and Pasture Land being positive. The Pasture/Hay conclusion came from the model that predicted extreme values for dissolved oxygen, so it is fair to conclude that there is more likely a positive relationship with grasslands and dissolved oxygen concentrations. This positive relationship may be due to a better soil structure beneath the grasslands due to the abundance of humic matter leading to larger soil pore space.

Grass and Pasture Land was also found to be significant in the most complex binned land class model. This landuse was found significant by itself and interacted with dissolved oxygen, as well as, interacted with dissolved oxygen and the vulnerability status. The interaction of Grass and Pasture Land for this model with dissolved oxygen might indicate that there is more going on than a simple direct relationship between the amount of grassland and observed dissolved oxygen values. This is reiterated by Grass and Pasture Lands' inclusion in the most complex term of all the models, although conclusion about this model should be taken with restraint as this model had some outrageous predictive values.

Conclusions

There is a relationship with landuse and nitrogen loading. Areas with heavy application of fertilizer, like that of cropland, show increased nitrogen loading and a corresponding increase in denitrification. This relationship is also dependent upon how connected the aquifer is to the surface and its respective landuse. Increasing loading in general leads to increases in denitrification. The opposite trend in loading and denitrification is observed for forestland.

Cropland's isotopic signature of "lighter" nitrogen was not evident in this study, but "heavier" isotopes were observed for low intensity developed land. The lack of the observed signature for cropland is likely due to the preferential use of lighter isotopes in biologically mediated processes. As for the relationship of heavier isotopes to low intensity developed land, this may indicate the presence of wastewater treatment facilities in these lands. Although, there was no observed relationship between source nitrate and wastewater effluent or reuse values which could be attributable to the small sample size.

This study shows that there are relationships with landuse and how much nitrogen is being removed from the water column, but does not take into account what future landuse changes have on these rates. If a landuse changes it may take anywhere from 3 – 35 years to reflect that change based off water age dating in the Upper Floridan Aquifer (Katz 2004). Furthermore, the residency of this water within these aquifers is thought to be somewhere between 10 – 20 years (Katz et al. 2001).

Looking forward it is important to note that the ability of these aquifers to remove nitrogen may become more and more limited due to inputs of saltwater. A study by Rivett and colleagues showed that as salinity rises denitrification rates are shown to decrease although not stop entirely (Rivett et al. 2008). Furthermore, saltwater intrusion has already been identified on the West coast of Florida though upward leeching from brackish aquifers (Schmerge 2001). These effects, along with sea level rise, may have implications for how and if nitrogen will be removed from the water column.

This study was conducted with regards to complete denitrification, but does not take into account speciation of the nitrogen removed. One important consideration for future management is how increased denitrification may lead to increased N_2O emissions, a much more potent greenhouse gas than that of CO_2. One important observation is that although cropland is seen to have a positive influence on denitrification, the corresponding nitrogen isotopes associated with these lands seemed to be missing, indicating that a lot of this nitrogen is off gassed from the aquifer. A conversion of more forestland (low nitrogen loading) to agriculture will likely lead to the creation of more N_2O from the aquifer in addition to off gassing of the fields themselves.

References

Aravena, R., M. L. Evans, and J. A. Cherry. 1993. Stable Isotopes of Oxygen and Nitrogen in Source Identification of Nitrate from Septic Systems. Ground Water 31:180–186.

Arthur, J. D., A. E. Baker, J. R. Cichon, A. R. Wood, and A. Rudin. 2005. Florida Aquifer Vulnerability Assessment (FAVA): Contamination potential of Florida's principal aquifer systems. Tallahassee, Florida.

Bateman, A. S., and S. D. Kelly. 2007. Fertilizer nitrogen isotope signatures. Isotopes in environmental and health studies 43:237–47.

Boyer, D. G., and G. C. Pasquarell. 1995. NITRATE CONCENTRATIONS IN KARST SPRINGS IN AN EXTENSWELY GRAZED AREA. Journal of the American Water Resources Association 31:729–736.

Branham, B., E. Miltnerr, and E. Spring. 1995. Potential Groundwater Contamination from Pesticides and Fertilizers Used on Golf Courses. US Green Section Record:33–37.

Canfield, D. E., A. N. Glazer, and P. G. Falkowski. 2010. The evolution and future of Earth's nitrogen cycle. Science (New York, N.Y.) 330:192–6.

Canty, A., and B. Ripley. 2013. boot: Bootstrap R (S-Plus) Functions.

Cole, M. L., K. D. Kroeger, J. W. Mcclelland, and I. Valiela. 2006. Effects of Watershed Land use on Nitrogen Concentrations and δ15 Nitrogen in Groundwater. Biogeochemistry 77:199–215.

Flipse, W., and F. Bonner. 1985. Nitrogen-�Isotope Ratios of Nitrate in Ground Water Under Fertilized Fields, Long Island, New York. Ground Water 23:59–67.

Ford, D., and P. Williams. 1989. Karst Geomorphology and Hydrology. Page 87 The Geographical Journal. Unwin Hyman, London, U.K.

Fox, J., and S. Weisberg. 2011. An R Companion to Applied Regression. Sage, Thousand Oaks, CA.

Fry, J., G. Xian, S. Jin, J. Dewitz, C. Homer, N. Herold, and J. Wickham. 2011. Completion of the 2006 National Land Cover Database for the Conterminous United States. Pages 858–864 Photogrammetric Engineering & Remote Sensing.

Galloway, J. N., J. D. Aber, J. W. Erisman, S. P. Seitzinger, W. Howarth, E. B. Cowling, B. J. Cosby, and J. A. N. Willem. 2010. The Nitrogen Cascade 53:341–356.

Hancock, P. J. 2002. Human impacts on the stream-groundwater exchange zone. Environmental management 29:763–81.

Heffernan, J. B., a. R. Albertin, M. L. Fork, B. G. Katz, and M. J. Cohen. 2012. Denitrification and inference of nitrogen sources in the karstic Floridan Aquifer. Biogeosciences 9:1671–1690.

Jordan, S. J., J. Stoffer, and J. A. Nestlerode. 2010. Wetlands as Sinks for Reactive Nitrogen at Continental and Global Scales: A Meta-Analysis. Ecosystems 14:144–155.

Katz, B. G. 2004. Sources of nitrate contamination and age of water in large karstic springs of Florida. Environmental Geology 46:689–706.

Katz, B. G., J. K. Böhlke, and H. D. Hornsby. 2001. Timescales for nitrate contamination of spring waters, northern Florida, USA. Chemical Geology 179:167–186.

Katz, B. G., D. W. Griffin, and J. H. Davis. 2009. Groundwater quality impacts from the land application of treated municipal wastewater in a large karstic spring basin: chemical and microbiological indicators. The Science of the total environment 407:2872–86.

Körner, H., and W. G. Zumft. 1989. Expression of denitrification enzymes in response to the dissolved oxygen level and respiratory substrate in continuous culture of Pseudomonas stutzeri. Applied and environmental microbiology 55:1670–6.

Korom, S. F. 1992. Natural denitrification in the saturated zone: A review. Water Resources Research 28:1657–1668.

Kreitler, C. W. 1979. Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. Journal of Hydrology 42:147–170.

Lenat, D. R., and J. K. Crawford. 1994. Effects of land use on water quality and aquatic biotia of three North Carolina Piedmont streams. Hydrobiologia:185–199.

Milburn, E. A. 2007. THE EFFECTS OF DREDGING ON DISSOLVED OXYGEN IN AGRICULTURAL WATERWAYS IN KING COUNTY, WASHINGTON. Washington State University.

Multi-Resolution Land Characteristics Consortium. 2006. National Land Cover Database 2006. http://www.mrlc.gov/nlcd06_leg.php.

Panno, S. V., K. C. Hackley, H. H. Hwang, and W. R. Kelly. 2001. Determination of the sources of nitrate contamination in karst springs using isotopic and chemical indicators. Chemical Geology 179:113–128.

Petitta, M., D. Fracchiolla, R. Aravena, and M. Barbieri. 2009. Application of isotopic and geochemical tools for the evaluation of nitrogen cycling in an agricultural basin, the Fucino Plain, Central Italy. Journal of Hydrology 372:124–135.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rivett, M. O., S. R. Buss, P. Morgan, J. W. N. Smith, and C. D. Bemment. 2008. Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. Water research 42:4215–32.

Schmerge, D. 2001. Distribution and Origin of Salinity in the Surficial and Intermediate Aquifer Systems , Southwestern Florida Distribution and Origin of Salinity in the Surficial and Intermediate Aquifer Systems , Southwestern Florida. Tallahassee, Florida.

Seiler, K., and I. Vomberg. 2004. Denitrification in a karst aquifer with matrix porosity. Pages 311–319 *in* R.-Z. Xi, K.-P. Seiler, and W.-Z. Gu, editors. Research Basins and Hydrological Planning. Neuherberg, Germany.

Spalding, R. F., M. E. Exner, C. W. Lindau, and D. W. Eaton. 1982. Investigation of sources of groundwater nitrate contamination in the Burbank-Wallula area of Washington, U.S.A. Journal of Hydrology 58:307–324.

Tihansky, A. B., and L. A. Knochenmus. 2001. Karst Features and Hydrogeology in West-central Florida — A Field Perspective. Tampa, Florida.

Vitousek, P. 1982. Nutrient Cycling and Nutrient Use Efficiency. The American Naturalist 119:553–572.

Wassenaar, L. I. 1995. Evaluation of the origin and fate of nitrate in the Abbotsford Aquifer using the isotopes of15N and18O in NO3−. Applied Geochemistry 10:391–405.

Wolkovich, E. M., D. a. Lipson, R. a. Virginia, K. L. Cottingham, and D. T. Bolger. 2010. Grass invasion causes rapid increases in ecosystem carbon and nitrogen storage in a semiarid shrubland. Global Change Biology 16:1351–1365.

Appendix

Appendix 1 – Springsheds Created for this Study

De Leon, Gemini, Charles, Lafayette Blue, Little River, Peacock, Rock Bluff, Running, Telford, Trail, Pothole.

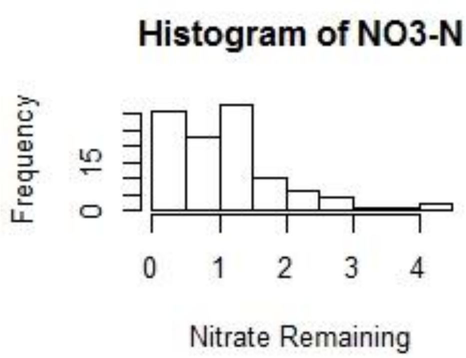
Appendix 2 – Comparison of Landuse for Total Dataset and Subset by Springshed

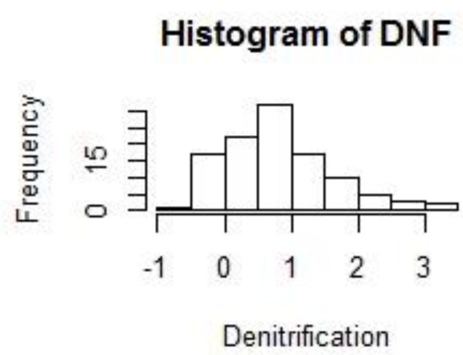
T Test for Study Sites (Springs) and Subset by Springsheds

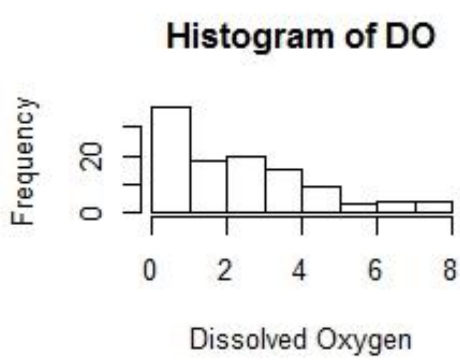
	Cultivated Crops	Developed, Open Space	Developed, Low Intensity	Open Water	Shrub/ Scrub#	Total Forest	Total Wetlands	Grass and Pasture Land	Other	Total Area
t statistc	1.18	2.3467	1.9467	2.0768	3.2079	2.0316	1.5549	2.6356	1.9784	2.3262
p-value	0.2401	0.02041	0.05367	0.03973	0.00225	0.04417	0.1223	0.009391	0.04994	0.02151

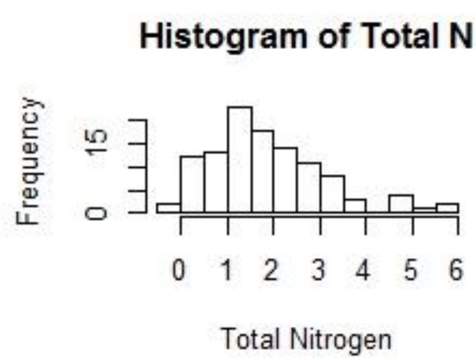
#Welch's T-test preformed due to unequal variance

Appendix 3 – Histograms of Water Quality Parameters

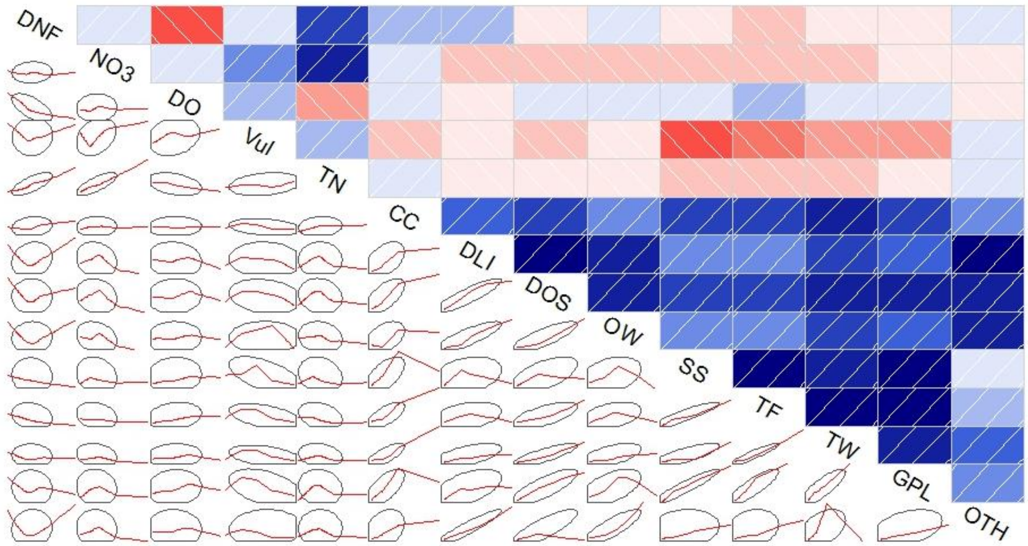








Appendix 4 – A Correlogram of the Data for Analysis with Binned Landuse Classes



DNF – Denitrification NO3 – Nitrate DO – Dissolved Oxygen Vul – Vulnerability TN – Total Nitrogen CC – Cultivated Crops DLI – Developed, Low Intensity DOS – Developed, Open Space OW – Open Water SS – Shrub/Scrub TF – Total Forest TW – Total Wetlands GPL – Grass and Pasture Land OTH – Other

Appendix 5 - LULC Descriptions

"Developed, Open Space - Includes areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20 percent of total cover. These areas most commonly include large-lot single-family housing units, parks, golf courses, and vegetation planted in developed settings for recreation, erosion control, or aesthetic purposes.

Developed, Low Intensity -Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 20-49 percent of total cover. These areas most commonly include single-family housing units.

Developed, Medium Intensity - Includes areas with a mixture of constructed materials and vegetation. Impervious surfaces account for 50-79 percent of the total cover. These areas most commonly include single-family housing units.

Developed, High Intensity - Includes highly developed areas where people reside or work in high numbers. Examples include apartment complexes, row houses and commercial/industrial. Impervious surfaces account for 80 to100 percent of the total cover.

Cultivated Crops - Areas used for the production of annual crops, such as corn, soybeans, vegetables, tobacco, and cotton, and also perennial woody crops such as orchards and vineyards. Crop vegetation accounts for greater than 20 percent of total vegetation. This class also includes all land being actively tilled.

Woody Wetlands - Areas where forest or shrub land vegetation accounts for greater than 20 percent of vegetative cover and the soil or substrate is periodically saturated with or covered with water.

Emergent Herbaceous Wetlands - Areas where perennial herbaceous vegetation accounts for greater than 80 percent of vegetative cover and the soil or substrate is periodically saturated with or covered with water.

Grassland/Herbaceous - Areas dominated by grammanoid or herbaceous vegetation, generally greater than 80% of total vegetation. These areas are not subject to intensive management such as tilling, but can be utilized for grazing.

Pasture/Hay - Areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops, typically on a perennial cycle. Pasture/hay vegetation accounts for greater than 20 percent of total vegetation.

Barren Land (Rock/Sand/Clay) - Barren areas of bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other accumulations of earthen material. Generally, vegetation accounts for less than 15% of total cover.

Shrub/Scrub - areas dominated by shrubs; less than 5 meters tall with shrub canopy typically greater than 20% of total vegetation. This class includes true shrubs, young trees in an early successional stage or trees stunted from environmental conditions.

Open Water - areas of open water, generally with less than 25% cover of vegetation or soil.

Deciduous Forest - areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species shed foliage simultaneously in response to seasonal change.

Evergreen Forest - areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. More than 75% of the tree species maintain their leaves all year. Canopy is never without green foliage.

Mixed Forest - areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. Neither deciduous nor evergreen species are greater than 75% of total tree cover"(Multi-Resolution Land Characteristics Consortium 2006).

Total Forest – All forest layers combine into one overarch land cover.

Grass and Pasture Land – Area from both Grassland/Herbaceous and Pasture/Hay land covers.

Other – The combination of Barren Land; Developed, High Intensity; and Developed, Low Intensity. These classes were binned together due to their lack of representation in the dataset.